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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003903296 for a patent by RAUSTECH PTY LTD as filed on 30 June 2003.

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Eighth day of July 2004

JULIE BILLINGSLEY  
TEAM LEADER EXAMINATION  
SUPPORT AND SALES



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**AUSTRALIA  
PATENTS ACT 1990**

**PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:  
"CHEMICAL COMPOSITIONS OF MATTER"**

**This invention is described in the following statement:-**

This invention relates to novel emulsions and methods of use of such emulsions in the manufacture of solid phase arrays using such emulsions.

The invention will be generally discussed in relation to manufacture and use of

5 emulsions which are useful for manufacture of DNA arrays of the type generally known as DNA chips on substrates particularly planar substrates but the use of the invention is not limited to that particular application but has wider ramifications and the invention is not intended to be limited to the manufacture of such DNA chips.

10 In its broadest form the invention relates to the emulsion mediated spatially defined deposition of any of a wide variety of chemical substances onto a surface. Substances may include, but are not limited to, coloured materials, dyes, polymers, catalysts, anti-wetting agents and pigments, etching chemicals, layerings and reagents for de-blocking, blocking, derivatisation and activation of solid phase chemical groups.

15 Arrays can include deoxyribonucleic acids (DNA), peptides, peptidenucleic acids (PNA), ribonucleic acids (RNA) and other solid phase chemical arrays and arrays assembled by combinatorial chemistry.

In general the manufacture of DNA chips involves the selective and sequential

20 addition onto a substrate, of molecular units each with a protective group which is removed when the next molecular unit is to be added. One such method of manufacturing DNA arrays uses a process known as the phosphoramidite process which uses a trityl group or derivatives of the trityl group as the protective group. The invention is not limited to this process but will be discussed with respect to it.

25 The phosphoramidite process is a repetitive four stage process (deprotection, coupling, capping and oxidation) for the chemical synthesis of polymers particularly sequences of DNA oligonucleotides to form portions of DNA.

30 In the phosphoramidite process, a portion of DNA in single stranded form is built up by the sequential addition in predetermined order of any one of the four nucleotides (in phosphoramidite form) being the four components which make up DNA, the A,

T, G and C nucleotides. Each nucleotide has a chemically removable protecting group on it. A chemical reagent known as a de-protecting agent removes the protecting group exposing a reactive hydroxyl group and in the next stage a nucleotide (in phosphoramidite form) is coupled to the growing DNA string. The 5 next stage is a capping step where any DNA strings which were de-protected but to which a nucleotide was not coupled are permanently capped to prevent unwanted addition of nucleotides from adding to that molecule in later coupling steps. In the fourth and final step, oxidation of the newly formed inter-nucleotide phosphite linkage is carried out to convert the linkage to a phosphotriester.

10

In the manufacture of DNA arrays, a number of different sequence DNA strands are built up on a substrate to enable later bio-chemical analysis to take place. In this process it is necessary to selectively de-protect various portions of the array and it is particularly to this selective de-protecting that one particular embodiment of the 15 present invention is directed.

Selective de-protecting by direct light-activated chemistry or photo-removable de-protecting techniques has been developed but these are somewhat inefficient resulting in short and impure solid phase oligodeoxynucleotides in rather large unit 20 feature probe sizes of 20 to 50 microns and it is an object of this invention to provide a more efficient chemical de-protecting process.

The applicant has surprisingly found that by the use of electrically charged emulsions which include the chemical de-protecting agent in the discontinuous 25 phase and which are selectively deposited on predefined areas of a planar or other shaped substrate under the influence of an electric field, then more accurate, localised and efficient de-protecting may be possible.

In one form therefore, although this may not necessarily be the only or broadest 30 form, the invention is said to reside in a composition of matter including a liquid continuous phase and a liquid discontinuous phase which is substantially immiscible

in the continuous phase, characterised by the continuous phase having a high electrical volume resistivity and the discontinuous phase being electrically charged.

In further form the invention is said to reside in a composition of matter including a  
5 liquid continuous phase, a liquid discontinuous phase which is substantially immiscible in the continuous phase and a surfactant, characterised by the continuous phase having a high volume resistivity, the discontinuous phase being electrically charged and the surfactant being selected to not significantly reduce the volume resistivity of the continuous phase.

10 The term "not significantly reduce the volume resistivity" is intended to mean that the volume resistivity of the continuous phase of the emulsion is not reduced to such an extent that the electrical charge on the discontinuous phase is compromised to the extent that the discontinuous phase will not deposit under the influence of an electric  
15 field.

It may be noted, too, that the choice of discontinuous phase or any of its components should be such that it does not significantly partition into the continuous phase and thereby neither significantly reducing the volume resistivity of the continuous phase  
20 nor imparting chemical reactivity to the chemical phase.

Preferably the surfactant is one which has a first part which is compatible with the continuous phase and a second part which is compatible with the discontinuous phase.

25 In an alternative form the invention is said to reside in an emulsion including a continuous phase, a discontinuous phase which is immiscible in the continuous phase, and a surfactant, the surfactant having a first part which is compatible with the continuous phase and a second part which is compatible with the discontinuous  
30 phase, characterised by the continuous phase having a high volume resistivity, the discontinuous phase being electrically charged and the surfactant being selected to not significantly reduce the volume resistivity of the continuous phase.

As discussed above the continuous phase is comprised of a liquid which is electrically insulative and although the characteristics of a particular system would have to be determined empirically it is expected that such a liquid would preferably

5 have a volume resistivity of approximately  $1 \times 10^6$  ohm-cm or greater.

The continuous phase may be selected from hydrocarbons such as hexane, cyclohexane, iso-octane, heptane, aromatic hydrocarbons and isodecane and commercially available mixtures of hydrocarbons such decalin, as the Isopars<sup>TM</sup> and

10 Norpars<sup>TM</sup> made by Exxon. The continuous phase may also be selected from fluoro-chemicals including fluoro-carbon compounds. These fluoro-chemicals generally comprise from 2 to 16 carbon atoms and include, but are not limited to, linear, cyclic or polycyclic perfluoroalkanes, bis(perfluoroalkyl)alkenes, perfluoroethers, perfluoroamines, perfluoroalkyl bromides and perfluoroalkyl chlorides such as the

15 Fluorinerts<sup>TM</sup> made by 3M. The continuous phase may also be selected from silicone fluids such as polyphenylmethyl siloxanes, dimethyl polysiloxanes, polydimethyl siloxanes, cyclic dimethyl siloxanes and the like.

The continuous phase may also be a gel or highly viscous liquid.

20 The discontinuous phase may be aqueous or non-aqueous. Where the discontinuous phase is non-aqueous it should be immiscible or substantially insoluble in the continuous phase.

25 The discontinuous phase may be a reagent such as a chemical de-protecting agent or it may be a solvent which carries the active chemical reagent. Alternatively the active chemical reagent may be a solid or insoluble liquid dispersed in the discontinuous phase.

30 The emulsions according to the invention may also include charge control agents such as ionic or zwitterionic compounds selected from metallic soaps wherein the metals include: barium, calcium, magnesium, strontium, zinc, cadmium, aluminium,

gallium, lead, chromium, manganese, iron, nickel, and cobalt and the acid portion is provided by a carboxylic acid of generally but not limited to at least 6 carbon atoms, e.g., caproic acid, octanoic (caprylic) acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linolic acid, erucic acid, tallitic acid, resinic acid,

5 naphthenic acid, succinic acid and the like. Examples of metallic soaps include: aluminium tristearate, aluminium distearate, barium, calcium, lead and zinc stearates; cobalt, manganese, lead and zinc linoleates; aluminium, calcium and cobalt octoates; calcium and cobalt oleates; zinc palmitate; calcium, cobalt, manganese, lead and zinc naphthenates; calcium, cobalt, manganese, lead and zinc resinates and the

10 like. Other suitable charge directors may include nonpolar liquid soluble ionic or zwitterionic charge director compounds such as sodium dioctylsulfosuccinate, zirconium octanoate and metallic soaps such as copper oleate. The charge control agent may also be lecithin and alkyl succinimide. Where the continuous phase is a fluorochemical the charge control agent may include a fluorine analogue of the above

15 compounds.

The additional components in the discontinuous phase may be bio-active agents, reagents and reactants such as acids and bases, blocking and de-blocking chemicals and derivitisation chemicals whether organic or inorganic, pharmaceuticals or dyes

20 or pigments.

An emulsion according to this invention may have a continuous phase which is present in the range of about 40 to 99.99 per cent by volume, a discontinuous phase which is present in a range of from about 0.01 to 60 per cent by volume, optionally a surfactant which is present in a range of about 0.01 to 20 per cent by weight and a charge control agent which is present in a range of 0.01 to 10 per cent by weight.

For some application the emulsions may be manufactured immediately prior to use and in such situations the surfactant may not be necessary.

30 Throughout this specification the term emulsions is used to indicate emulsions, mini-emulsions and micro-emulsions. Hence the emulsions according to this

invention may be true emulsions, that is, emulsions which are formed by the input of mechanical energy such as by shaking, stirring or the like. Emulsions may be manufactured using a device such as a Sonicator, Ultra-Turrax or a Microfluidiser.

Alternatively the emulsions may be mini-emulsions which form with the application

5 of more energy than for a standard emulsion. Alternatively the emulsions may be micro-emulsions which form substantially spontaneously provided the correct conditions of temperature and chemical composition are present. Emulsions may have a droplet size of from about 100 microns down to 0.2 microns, mini-emulsions may have a droplet size from 1000 nanometres down to about 50 nanometres and

10 micro-emulsions may have droplet sizes of from about 200 nanometres down to 1 nanometre. It will be noted that there is no hard and fast rule on the size ranges for each. Size ranges will depend upon the composition of each of the phases and the surfactant used and the method of preparation.

15 For this invention the particles or droplets of emulsion may range in size from 100 microns downwards depending upon the type of emulsion and the applications to which the emulsion is to be applied. Preferably in the case of emulsions for chemical de-protecting in the phosphoramidite process the emulsions may have a size range from 50 microns down to 20 nanometres.

20 Where the emulsion of the present invention is to be applied for the chemical de-protecting step of the formation of a DNA array, the invention may be said to reside in a composition being an emulsion including a continuous phase comprising an insulative liquid and a discontinuous phase comprising a non-aqueous or aqueous solvent and a chemical de-protecting reagent in solution in the non-aqueous or aqueous solvent, and wherein the continuous phase has a high volume resistivity and the discontinuous phase is electrically charged.

30 In an alternative embodiment where the emulsion of the present invention is to be applied for the chemical de-protecting step of the formation of a DNA array, the invention may be said to reside in a composition being an emulsion including a continuous phase comprising an insulative liquid, a discontinuous phase comprising

a non-aqueous or aqueous solvent and a chemical de-protecting reagent in solution in the non-aqueous or aqueous solvent and a surfactant, the surfactant having a first part which is compatible with the continuous phase and a second part which includes a group which is compatible with the discontinuous phase, and wherein the

5 continuous phase has a high volume resistivity and the discontinuous phase is electrically charged and the surfactant being selected to not significantly reduce the volume resistivity of the continuous phase.

Preferably where the continuous phase is a fluorochemical the fluorochemical is a

10 perfluoro-carbon such as perfluoro-octanate, linear, cyclic or polycyclic perfluoroalkanes, bis(perfluoroalkyl)alkenes, perfluoroethers, perfluoroamines, perfluoroalkyl bromides and perfluoroalkyl chlorides such as the Fluorinerts<sup>TM</sup> made by 3M.

15 The continuous phase may alternatively be a silicone fluid or an organic liquid, such as a hydrocarbon oil selected from hexane, cyclohexane, iso-octane, heptane, aromatic hydrocarbons, decalin and isodecane and commercially available mixtures of hydrocarbons such as the Isopars<sup>TM</sup> and Norpars<sup>TM</sup> made by Exxon.

20 Preferably where the continuous phase is a fluorochemical the surfactant is a fluorochemical-hydrocarbon di-block molecule having a fluorophilic part and a lipophilic part. These compounds may also be referred to as amphiphiles. Examples of these are perfluorocarbon-propoxypropylene, fluoro-alkyl citrate, perfluoroalkyl-alkylene mono- or di- morpholinophosphate and fluorinated phospholipids,

25 alcohols, polyols or polyhydroxylated or aminated derivatives including amine oxides and amino acid derivatives.

The fluorinated surfactants may also be associated with hydrogenated, non-ionic, anionic, cationic or zwitterionic surfactants. Such hydrogenated surfactants include,

30 for example, phospholipids, copolymers of the polyoxyethylene polyoxyethylenepolyoxypropylene type and polyoxyethylene sorbitan esters.

The surfactants for the emulsion where the discontinuous phase is water may be selected from non-ionic, anionic, cationic or zwitterionic surfactants.

There may also be used combinations of the various surfactants discussed above.

5

The emulsion according to this form of the invention may further include a charge control agent of the type discussed above. The charge control agent may be sodium dioctylsulfosuccinate, zirconium octoate and metallic soaps such as copper oleate or be lecithin or alkyl succinimide. In some embodiments the function of the charge

10 control agent may be provided by the surfactant or may be intrinsic to the emulsion droplets carrying a chemical substance, eg an acid for de-protection.

As discussed above it may be noted that the emulsions according to this embodiment of the invention may be true emulsions or mini-emulsions, that is, emulsions which 15 are formed by the input of mechanical energy such as by shaking, stirring or the like. Alternatively the emulsions may be micro-emulsions which form substantially spontaneously provided the correct conditions of temperature and chemical composition are present.

20 Alternatively the fluorocarbon of this embodiment may be replaced by an alternative compound such as a silicone fluid and hence the surfactant would be selected from a compound which had a silicophilic part.

25 The emulsion of the present invention may be used for a range of solid phase chemical reactions where it is desired to selectively spatially react on a substrate.

In one embodiment the emulsion may be used in a method of forming a solid phase chemical array on a substrate using a stepwise reaction process, the method including the steps of:

30 (a) defining at least one region on the substrate by forming an electric or electrostatic charge on that region which is different from the electric or

electrostatic charge on other regions of the substrate such as by formation of an electrostatic image thereon,

5           (b) applying an emulsion to the substrate, the emulsion having an electrically charged discontinuous phase and a chemical reagent carried in or comprising the discontinuous phase,

10          (c) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electric field on the region and optionally by the use of a bias voltage,

             (d) causing a chemical reaction in the at least one region,

15          (e) removing the emulsion, and

             (f) carrying out subsequent steps of the stepwise reaction process.

In an alternative embodiment it may be used in a method of forming a DNA array on a substrate using a stepwise coupling process with a chemical de-protecting step prior to each coupling step, the method including the steps of:

15          (g) defining at least one region on the substrate by forming an electric or electrostatic charge on that region which is different from the an electric or electrostatic charge on other regions of the substrate such as by formation of an electrostatic image thereon,

20          (h) applying an emulsion to the substrate, the emulsion having an electrically charged discontinuous phase and a chemical de-protecting reagent carried in the discontinuous phase as discussed above,

             (i) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electric field on the region and optionally by the use of a bias voltage,

25          (j) causing chemical de-protecting in the at least one region,

             (k) removing the emulsion, and

             (l) carrying out subsequent steps of the stepwise coupling process.

30          The subsequent steps of the stepwise coupling process may be such as those that are carried out in the standard phosphoramidite chemistry for synthesis of

oligodeoxynucleotides although as discussed earlier the invention is not limited to this particular chemistry.

It will be realised that the process as discussed above may be repeated a sufficient 5 number of times to synthesise selected oligonucleotides of any sequence in a predetermined spatial order, position and length on the substrate.

The substrate may be any insulative or dielectric material which is able to hold an electric charge for sufficient time for attracting the discontinuous phase of the 10 emulsion to the at least one preselected region. The substrate may include dielectrics such as glass, plastics materials or the like and alternatively may include photo conductors such as zinc oxide, selenium and the like.

The step of defining at least one region on the substrate by forming an electric or 15 electrostatic charge on that region may include the step of charge reversal to thereby provide an enhanced electric field in the selected region.

The formation of the an electric or electrostatic charge may be by electrostatic means such as wherein the substrate is a photoconductor and the formation of the 20 electrostatic field is by electrostatic or other charging and then selective discharge by illumination. Preferably the illumination may not include radiation in the short ultraviolet region as this may cause damage to the DNA molecule. For the assembly of other chemical chips or arrays, however, UV radiation may be used.

25 In one preferred embodiment, the stepwise synthesis process is the phosphoramidite process which uses chemical de-protecting, namely removal of a trityl group but the invention is not so limited but may include other stepwise coupling or addition processes.

30 The step of removing the emulsion may include the step of neutralising any residual chemical de-protecting agent in the emulsion to prevent it from reacting in non-desired parts of the array.

As discussed above, the emulsion for use for the present invention comprises an electrically insulative continuous phase such as a fluorochemical, an aqueous or a non-aqueous discontinuous phase for instance a hydrocarbon oil which carries the

5 chemical de-protecting agent in it in solution, with preferably a surfactant and preferably a charge control agent.

Where the discontinuous phase is water the chemical de-protecting agent may be a weak organic acid such as acetic acid. Acetic acid may be present in a concentration  
10 of up to 80% by volume.

Where the discontinuous phase is a hydrocarbon oil, the chemical de-protecting agent may be a strong protic organic or inorganic acid.

15 The non-aqueous discontinuous phase which carries the chemical de-protecting agent in solution may be selected from acetone, acetonitrile, cyclohexanone, dibromomethane, dichloromethane (methylene chloride, DCM), trichloromethane, dimethyl formamide (DMF), dioxane, 1,2-dichloroethane (DCE), nitromethane, tetrahydrofuran, toluene, dimethyl formamide or mixtures of compounds such as  
20 isopropanol/methylene chloride, nitromethane/methanol, nitromethane/isopropanol, trichloromethane/methanol or isopropanol/methylene chloride. Other hydrocarbons such as decalin may also be used.

The chemical de-protecting agent may be a Lewis acid or a protonic acid. The Lewis  
25 acid may be selected from but not restricted to zinc bromide, titanium tetrachloride, and ceric ammonium nitrate while dilute protonic acids which can be used include, but are not limited to, dilute mineral acids, trichloroacetic acid (TCA), dichloroacetic acid (DCA), benzenesulphonic acid, trifluoroacetic acid (TFA), difluoroacetic acid, perchloric acid, orthophosphoric acid and toluenesulphonic acid. Other acids may  
30 include dodecylbenzene sulphonic acid and diphenyl acid phosphate.

- a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% (w/v) Atlox 4912 with respect to the discontinuous phase
- a 0.5% to 2.5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 2.5% (w/v) Atlox 4912 with respect to the discontinuous phase
- a 0.5% to 1% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 5% (w/v) Atlox 4912 with respect to the discontinuous phase
- a 0.5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 10% (w/v) Atlox 4912 with respect to the discontinuous phase.

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of Isopar G and:

- a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 5% (w/v) Triton X-100 with respect to the discontinuous phase.

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of Isopar G and:

- a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 5% (w/v) cetyltrimethylammonium bromide (CTAB) with respect to the discontinuous phase.

CTAB was purchased from Sigma Chemical Company.

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of Isopar G and:

- a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 5% (w/v) sodium dodecylsulphate (SDS) with respect to the discontinuous phase.

SDS was purchased from APS Ajax Finechem, Australia.

In a further experiment comparable results were obtained when the emulsion  
5 composition comprised a continuous phase of Isopar G and:

- a 0.5% to 1% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 5% (w/v) 1,4 bis (2-ethylhexyl) sulphosuccinate with respect to the discontinuous phase.

10 1,4 bis (2-ethylhexyl) sulphosuccinate (AOT) was purchased from Sigma Chemical Company.

In a further experiment comparable results were obtained when the emulsion  
composition comprised a continuous phase of Isopar G and:

15 • a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% to 10% (w/v) Q2-5200 with respect to the discontinuous phase.

Q2-5200 (DC-5200) is a comb polymer surfactant comprising a backbone of silicone  
20 and substituents of lauryl and polyethylene / polypropylene oxide groups. It is manufactured by Dow Corning.

**Water in Silicone Fluid**

In a further experiment comparable results were obtained when the emulsion  
25 composition comprised a continuous phase of Silicone Fluid 10 centipoise and:

- a 0.5% to 5% (v/v) aqueous discontinuous phase with disulphine blue and a surfactant concentration of 0.5% (w/v) 1,4 bis (2-ethylhexyl) sulphosuccinate with respect to the discontinuous phase.

30 Silicone fluid 10 centipoise is manufactured by Dow Corning.

**Water in fluorocarbons**

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included an acid and a substrate

5 comprising a zinc oxide photoconductor was charged with a negative pattern. The photoconductor was dip-coated with a solution of Butvar 72 (1% w/v) and the pH indicator methyl orange (at saturation) in cyclohexanone and dried at 55°C for 30 minutes:

10 The emulsion comprised the following;

Continuous phase	FC40	0.95ml
Discontinuous phase	Water	0.035ml
	Dichloroacetic acid	0.01ml
	FC134 (10% w/v in water)	0.005ml

15

FC40 is a fluorocarbon solvent manufactured by 3M.

Dichloroacetic acid was supplied by Aldrich Chemical Co, Inc.

FC134 is a fluoro-surfactant manufactured by 3M.

Methyl orange is a pH indicator dye manufactured by Kochlight.

20 Butvar 72 is polyvinyl butyral manufactured by Solutia.

It was found that the discontinuous phase incorporating the acid deposited only in the area of the charge pattern, giving a pH-dependent colour change from yellow to pink.

25

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC77.

The emulsion comprised the following;

Continuous phase	FC77	0.95ml
Discontinuous phase	Water	0.035ml
	Dichloroacetic acid	0.01ml

FC300                    0.005ml

FC77 is a fluorocarbon solvent manufactured by 3M.

FC300 is a fluoro-surfactant manufactured by 3M.

5

#### Water in toluene

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative

10 continuous phase and a discontinuous phase which included a dye and a substrate comprising a zinc oxide photoconductor which was charged with a negative pattern:

The emulsion comprised the following:

Continuous phase	Toluene	0.995ml
15 Discontinuous phase	Water	0.025ml
	Disulphine blue (20mg/ml aqueous)	0.025ml
	QC-5200 (10% w/v in toluene)	0.005ml

Toluene AR is a hydrocarbon supplied by BDH Chemicals Australia Pty Ltd.

20

It was found that the discontinuous phase incorporating the blue dye deposited only in the area of the charge pattern.

25 In a further experiment comparable results were obtained when the emulsion composition comprised a discontinuous phase incorporating Triton X-100 as surfactant.

The emulsion comprised the following;

Continuous phase	Toluene	0.995ml
30 Discontinuous phase	Disulphine blue (20mg/ml aqueous)	0.0025ml
	Triton X-100 (10% w/v in water)	0.0025ml

### Silicon Fluid in Fluorocarbon

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative

5 continuous phase and a discontinuous phase not including a dye and a substrate comprising a zinc oxide photoconductor was charged with a negative pattern:

The emulsion comprised the following:

Continuous phase	FC40	0.95ml
10 Discontinuous phase	Silicon fluid 50CS	0.05ml

Silicone fluid 50 centipoise is manufactured by Dow Corning.

It was found that the discontinuous phase deposited only in the area of the charge  
15 pattern, visible by a "wetting" effect on the photoconductor.

In a further experiment comparable results were obtained when the emulsion composition comprised a discontinuous phase incorporating Zonyl® TBC as surfactant.

20

The emulsion comprised the following:

Continuous phase	FC40	0.95ml
Discontinuous phase	Silicon fluid 50CS saturated with TBC	0.05ml

25 Zonyl® TBC is a fluorotelomer intermediate manufactured by DuPont Chemicals.

It was found that the discontinuous phase deposited only in the area of the charge pattern, visible by a "wetting" effect on the photoconductor remaining after the continuous phase solvent had evaporated.

30

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC77.

The emulsion comprised the following:

Continuous phase	FC77	0.95ml
Discontinuous phase	Silicon fluid 50CS	0.05ml

5

It was found that the discontinuous phase deposited only in the area of the charge pattern, visible by a "wetting" effect on the photoconductor.

In a further experiment comparable results were obtained when the emulsion  
10 composition comprised a continuous phase of FC77 and a discontinuous phase incorporating Zonyl® TBC as surfactant.

The emulsion comprised the following:

Continuous phase	FC77	0.95ml
15 Discontinuous phase	Silicon fluid 50CS saturated with TBC	0.05ml

It was found that the discontinuous phase deposited only in the area of the charge pattern, visible by a "wetting" effect on the photoconductor.

20 **Toluene in Fluorocarbon solvent**

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included an acid and a substrate  
25 comprising a zinc oxide photoconductor was charged with a negative pattern. The photoconductor was dip-coated with a solution of Butvar 72 (1% w/v) and the pH indicator methyl orange (at saturation) in cyclohexanone, and dried at 55 degrees C for 30 minutes:

30 The emulsion comprised the following:

Continuous phase	FC40	0.89ml
Discontinuous phase	5% dichloroacetic acid (w/v) in toluene	0.1ml

F6H14 (1% in FC40, v/v) 0.01ml

F6H14 is a semifluorinated alkylalkane, 1-(perfluoro-n-hexyl) tetradecane, manufactured by Apollo Scientific Ltd, UK.

5

It was found that the discontinuous phase deposited only in the area of the charge pattern, giving a pH-dependent colour change from yellow to pink.

10 In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC40 and a discontinuous phase incorporating higher levels of F6H14 as surfactant.

The emulsion comprised the following:

Continuous phase	FC40	0.8ml
15 Discontinuous phase	5% (w/v) dichloroacetic acid in toluene	0.1ml
	F6H14 (1% in FC40, v/v)	0.1ml

It was found that the discontinuous phase deposited only in the area of the charge pattern, giving a pH-dependent colour change from yellow to pink.

20

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included a dye and a substrate comprising a zinc oxide photoconductor was charged with a negative pattern:

The emulsion comprised the following:

Continuous phase	FC40	0.9ml
Discontinuous phase	0.2% ethyl red in toluene (w/v)	0.05ml
30	5% Zonyl® FTS in toluene (w/v)	0.05ml

Ethyl Red is 2-[p-diethylaminophenylazo]-benzoic, supplied by Sigma Chemical Co.

cyclohexanone (dried at 55 degrees C for 30 minutes) was charged with a negative pattern.

The emulsion comprised the following:

5	Continuous phase	FC40	0.998ml
	Discontinuous phase	5% (w/v) oxalic acid, 0.002% (w/v) Rhodamine B both in DMSO	0.001ml
		3% FC134 in DMSO	0.001ml

10 Oxalic acid was supplied by BDH Laboratory Supplies, UK

DMSO was supplied by Ajax FineChem, Australia

It was found by observing the coverslip under fluorescence microscopy that the discontinuous phase deposited only in the area of the charge pattern, giving a

15 brilliant pink colouration.

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative

20 continuous phase and a discontinuous phase which included a dye and polymer dissolved in the discontinuous phase so as to protect the dye from both water and air during and after deposition. A substrate comprising a zinc oxide photoconductor was charged in its entirety then covered with a mask before exposure to light so as to form a charge pattern that was a replica of the mask.

25

The emulsion comprised the following:

Continuous phase	FC40	0.99ml
Discontinuous phase	1% Butvar 72 (w/v), 0.1% (w/v) crystal violet in DMSO	0.005ml
30	1% (w/v)Atlox 4912 in DMSO	0.005ml

Crystal violet was supplied by George T. Gurr, Searle Scientific Services, Bucks, UK

0.002% Rhodamine B both in	
ethylene glycol (w/v)	0.001ml
F6H14	0.01ml

5 Rhodamine B was supplied by Sigma Chemical Co.

It was found by observing the coverslip under fluorescence microscopy that the discontinuous phase deposited only in the area of the charge pattern, giving a brilliant pink colouration.

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In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC40 and a discontinuous phase incorporating F6H14 as surfactant at higher levels.

15 The emulsion comprised the following:

Continuous phase	FC40	0.949ml
Discontinuous phase	5% dichloroacetic acid and 0.002% Rhodamine B both in ethylene glycol (w/v)	0.001ml
	F6H14	0.05ml

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In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC40 and a discontinuous phase incorporating F6H14 as surfactant at even higher levels.

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The emulsion comprised the following:

Continuous phase	FC40	0.749ml
Discontinuous phase	5% dichloroacetic acid and 0.002% Rhodamine B both in ethylene glycol (w/v)	0.001ml
	F6H14	0.25ml

### Nitrobenzene in fluorocarbon

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative

5 continuous phase and a discontinuous phase which included a fluorescent dye and a substrate comprising a glass microscope coverslip dip-coated with 1% Butvar 72 in cyclohexanone (dried at 55 degrees C for 30 minutes) was charged with a negative pattern.

10 The emulsion comprised the following:

Continuous phase	FC40	0.989ml
Discontinuous phase	5% dichloroacetic acid (v/v), 5% VTACL (w/v), Rhodamine 6G at saturation all in nitrobenzene	0.01ml
15	F6H14	0.001ml

Rhodamine 6G was supplied by George T. Gurr (a division of Baird & Tatlock, Essex, UK).

15 VTACL is a low molecular weight fraction of vinyl toluene acrylate (Pliolite)  
20 manufactured by Goodyear.

It was found by observing the coverslip under fluorescence microscopy that the discontinuous phase deposited only in the area of the charge pattern, giving a brilliant pink colouration.

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### Dimethylsulphoxide (DMSO) in fluorocarbon

A further experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative  
30 continuous phase and a discontinuous phase which included a fluorescent dye and a substrate comprising a glass microscope coverslip dip-coated with 1% Butvar 72 in

It was found that the discontinuous phase deposited only in the area of the charge pattern not exposed to light, giving a violet colouration. Surface regions as small as 50 microns across, the smallest feature attempted were clearly defined by dye 5 deposition.

In a further experiment comparable results were obtained when the emulsion composition comprised a continuous phase of FC40 and a discontinuous phase incorporating a polymer, AOT as surfactant and the pink fluorescent dye Rhodamine 10 B.

The emulsion comprised the following:

Continuous phase	FC40	0.99ml
Discontinuous phase	1% Butvar 72 (w/v), 0.1% (w/v) Rhodamine B	
	both in DMSO	0.005ml
	10% (w/v) AOT in DMSO	0.005ml

It was found that the discontinuous phase deposited only in the area of the charge pattern not exposed to light, giving a fluorescent pink colouration.

Emulsions tested in the above experiments were prepared using ultrasonic generators. For the experiments only small volumes are required and also the cost of the perfluorocarbons used as the continuous phase in the nonaqueous emulsions are high, therefore equipment must be able to emulsify the smallest volumes that are 20 practically possible. The rule of thumb for the production of mechanical emulsions is that the higher the power input per unit volume the smaller the emulsion size also the lower the surfactant requirement. This is achieved by the following equipment. 25 Luke Ultrasonics Cleaning system: Power 360 watts, used for emulsification & slide cleaning. Equipment manufactured in Australia.

Sonics & Materials Vibra-cell Model-CV-17 Probe System: Power 600 watts, used for emulsification 1/4" probe, controlled power and timed output.

For commercial production of emulsions according to the invention continuous production may be desirable. Devices which may be used include:

An Ultra-Turrax unit with an inline unit dispersing unit (also known as a generator) catalogue numbers 25 KV- 25 F-JL that is capable of producing an emulsion 1-5

5 microns in size.

Microfluidizer M110-S from Microfluidics Corporation can generate up to 23, 000 psi in the emulsification chamber, with a sample size minimum of 14 ml. with >12 ml. recovery. This unit is capable of the production of nanometer size emulsions.

- 10 The Microfluidics unit is capable of producing the smallest size mechanical emulsions with the lowest amount of surfactant. This may be of great importance in the present invention as the surfactants in some applications can have effects on the electrical conductivity of the continuous phase.
- 15 Throughout this specification various indications have been given as to the scope of this invention but the invention is not limited to any one of these but may reside in two or more of these combined together. The examples are given for illustration only and not for limitation.
- 20 Throughout this specification and the claims that follow unless the context requires otherwise, the words 'comprise' and 'include' and variations such as 'comprising' and 'including' will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.
- 25 Dated this 30<sup>th</sup> day of June, 2003.

Raustech Pty Ltd  
By its Patent Attorneys  
MADDERNS



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